phosphate inside an organism with thio-phosphate such that modified nucleic acids are generated from their respective NTP pools. It does not attempt to address to what extent, i.e. for the purpose of stabilizing mRNA. The idea is that the modified nucleic acids may be a useful label to identify growing cells etc. (see description of preferred embodiments) particularly with sensitive approaches like in vivo NMR. This is a novel approach for non-radioactively labeling nucleic acids in cells.

The present invention provides several examples of how to modify the culture media for incorporation of thio-phosphate into nucleic acids including using an excess relative to any phosphate that may be present in the media. Similarly, the present invention indicates that in more complex systems there is a need to provide an excess of thio-phosphate relative to internal phosphate pools. While only one example was given for a complex organism it was not a simple test. The thio-phosphate had to be taken up during digestion from the media and incorporated into the intestinal DNA of the fish. This process no doubt exposed the compound to degradative conditions and yet it was possible to see substantial incorporation into intestinal DNA. It would presumably be much easier to get incorporation via injection into the blood or interstitial fluid.

The demonstration of modified DNA in the fish infers similar incorporation into RNA, as deoxynucleotides are synthesized from ribonucleotides via *Ribonucleotide reductase*. While not direct proof of incorporation into RNA in this instance, it is a logical conclusion. There is no doubt that by bathing cells with an excess of thio-phosphate one could modify at least some of the nucleic acids in the cells of a complex organism. Further work is required to develop optimal detection schemes and strategies for specific goals. With regard to specificity for incorporation into DNA, DNA synthesis must take place, i.e. growing cells and for incorporation into RNA cells must be viable but not necessarily growing. Thus one can presumably preferentially label RNA as opposed to DNA under the right conditions.

The parent application incorporated by way of reference and the present application have shown the incorporation of thio-phosphate in a variety of cell types and processes such as phage and plasmid DNA synthesis as well as RNA and genomic DNA synthesis in both prokaryotic and eukaryotic cells. The universal nature of nucleic acid synthesis supports the general utility of this approach for a wide variety of organisms. The diversity of species and systems to which the method has been easily applied also speaks to it general utility. The jump from

DNA synthesis during phage infection in E. coli to gold fish intestinal DNA synthesis couldn't be farther apart.

Claim 10

Once it was realized that the incorporation of thio-phosphate into RNA could lead to the stabilization of RNA, the next obvious question was whether or not it could be used to enhance protein synthesis. In contrast to nucleic acid synthesis protein synthesis is a bit more complicated. An optimal amount of substitution is required for maximal protein synthesis. This is expressed as an optimal ratio of thio-phosphate to phosphate. The question is why is this so? This does not appear to be due to difficulties with RNA processing as it was shown that yeast actin mRNA is correctly processed to a mature mRNA and that both bacterial and yeast cells are viable in the presence of 100% thio-phosphate. Another possibility is that cells have a limited capacity for protein synthesis. Cells are inherently restricted in size and only some proteins can be excreted which naturally sets limits on how much additional protein a cell can tolerate. After observing a ten fold increase in alkaline phosphatase protein expression in yeast the next obvious question was could an increase in total protein synthesis be detected. To do this total soluble protein levels were measured using a standard biruet reagent (last paragraph of experimental section II). Using either yeast or bacteria no increase in total soluble protein expression was observed for ratios of thio-phosphate to phosphate of 10-30% where optimal induction generally occurs. At higher concentrations total protein synthesis was reduced somewhat but this appeared to be due to a slight decrease in growth. Still, in some instances significant protein induction could be observed at higher ratios such as for alkaline phosphatase expression in yeast (table 1). This would suggest preferential translation to compensate for the slight reduction in total protein synthesis observed. Consistent with this idea is the inhibition of other yeast genes at higher levels namely the extracellular nucleases and lipase. The alkaline phosphatase gene is of bacterial origin and so presumably the mRNA for this gene is not as stable as other the mRNAs for the extracellularly excreted yeast enzymes.

The basis for enhanced protein expression using thio-phosphate in culture media is increased stability of mRNA in cells leading to the accumulation of more protein. This mechanism preferentially enhances unstable mRNAs relative to more stable mRNAs as unstable mRNAs have the most to gain. If protein synthesis becomes rate limiting then more stable mRNAs may be less competitive and protein synthesis could actually decrease for these mRNAs. This was actually observed for beta-galactosidase win E. coli here proteins synthesis was decreased under inductive conditions for the gene at a 30% ratio of thio-phosphate to phosphate. At a higher ratios the expression of beta-galactosidase recovered consistent with the idea that at this ratio it was more competitive for translation. Under conditions where the mRNA is known to be unstable (i.e. in the absence of inducer) it was possible to enhance the expression (experimental section II para 6) of beta-galactosidase at a 50% ratio of thio-phosphate to phosphate. This is consistent with the claimed mechanism indicating the unstable mRNAs are preferentially enhanced at the expense of more stable mRNAs.

The examples in yeast and bacteria given above support the claimed mechanism which is clearly more than mere speculation. Another consideration is the example of actin mRNA in yeast which accumulated to a significant extent at 10 fold in 100% thio-phosphate substituted mRNA. You can envision quite a lot of mRNA in cells above normal, thereby restricting the cells capacity for protein translation.

I wish to declare and make a verified statement that the modified claims include no new matter as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b) or 1.825 (d).

Please under MPEP 707.07(j) the pro se applicant requests that if the Examiner finds patentable subject matter disclosed in this application, but feels that applicant's present claims are not entirely suitable, the Examiner draft one or more allowable claims for the applicant

Respectfully submitted,

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